bio-protocol Preprint

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Single cell library preparation

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An abbreviated version of this protocol was published in eLIFE in Jan 2020

Gene regulatory network reconstruction using single-cell RNA sequencing of barcoded genotypes in diverse environments

DOI: 10.7554/eLife.51254

Detailed protocol

Good afternoon-

1) BSA is an effective surfactant that suppresses clumping and improves pelleting without damaging cells.

2) 2kxg for 2 minutes. It may be necessary to remove 900ul and spin again.

3) Heat shock response would be swamped by osmotic & cell wall stress response.

4) RNAlater is saturated ammonium sulfate and will precipitate RNA in place, freezing the transcriptome and preventing changes during processing.

5) Yes; a small amount of background RNA is expected, but can be quantified after sequencing. We see fewer than 10 counts per barcode in non-cell droplets, which is a combination of cells that lyse prior to encapsulation, template switching, and sequencing error.

Thanks
-Chris

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Jackson, C. (2020). Single cell library preparation. Bio-protocol Preprint. bio-protocol.org/prep603.
2. Jackson, C. A., Castro, D. M., Saldi, G., Bonneau, R. and Gresham, D.(2020). Gene regulatory network reconstruction using single-cell RNA sequencing of barcoded genotypes in diverse environments. eLIFE. DOI: 10.7554/eLife.51254